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EXAMINER	
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ART UNIT	PAPER NUMBER
1642	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)
09/890,319	Dhellin et al
Examiner	**Art Unit**
Karen Canella	1642

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3 months* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☐ Responsive to communication(s) filed on _____ .

2a) ☐ This action is FINAL. 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under ***Ex parte Quayle***, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) *1-25* is/are pending in the application.

4a) Of the above, claim(s) _____ is/are withdrawn from consideration.

5) ☒ Claim(s) *1-3, 5-7, 11, 12, 17-19, 23, and 24* is/are allowed.

6) ☐ Claim(s) _____ is/are rejected.

7) ☒ Claim(s) *4, 8-10, 13-16, 20-22, and 25* is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☒ The specification is objected to by the Examiner.

10) ☒ The drawing(s) filed on *Jul 26, 2001* is/are a) ☐ accepted or b) ☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☒ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☒ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
3. ☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). *1*
4) ☐ Interview Summary (PTO-413) Paper No(s). _____
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

1. Claims 1-25 are pending and examined on the merits.

Specification

2. The use of multiple trademarks, for example, on page 5, lines 28-29, page 7, lines 14-16 and 21, page 9, lines 20 and 22, page 13, lines 8-10, page 14, lines 1-2, page 15, line 14, page 17, line 11, page 25, lines 2, 10, 15-17, 19 and 26, page 26, line 21, page 27, line 29, page 28, line 12, page 30, line 30, page 31, lines 8, 10-11, 13, 18-19, 21, 25 and 29, page 32, lines, 5, 6, 23 and 29, page 33, lines 24-25, page 34, lines 6, 10, 26 and 28, page 35, lines 1-2, 7, 13, 16 and 20, page 36, lines 7-8, 14 and 20, page 37, lines 1, 5, 11, 13 and 16-17, page 38, line 9-10, 13 and 19, page 39, lines 2, 8 and 14-15, page 40, lines 16, 18-19 and 27, page 41, lines 15 and 28, and page 43, lines 1 and 10, has been noted in this application. The trademarks should be capitalized wherever appearing and be accompanied by generic terminology.

Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

Appropriate correction is required.

Claim Objections

3. Claims 4, 8, 9, 10, 13, 14, 15, 16, 20, 21, 22 and 25 are objected to under 37 CFR 1.75(c) as being in improper form because multiple dependent claims may not serve as a basis for any other multiple dependent claim. See MPEP § 608.01(n). Accordingly, the claims have not been further treated on the merits.

Claim 5 is objected to because of the following informalities: the claim appears to have a part "b" and a part "c" without a part "a". Appropriate correction is required.

Claim Rejections - 35 USC § 112

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 2, 3, 6, 7, 11, 12, 18, 19, 23 and 24 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

(A)The term "strong" in claim 2 is a relative term which renders the claim indefinite. The term "strong" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The example of a strong anion exchanger give on page 6, lines 27-30 does not suffice to limit the metes and bounds of what defines"strong" as the example is set forth as a general, versus absolute, limitation.

(B) Claim 6 includes "(e.g. exosome)"; claim 11 includes "(e.g. exosome) and "(e.g. with exosomes)". Claim 19 includes "(e.g. bone marrow or peripheral blood)". It is unclear if the text within the parentheses is intended to limit the metes and bounds of the claim.

(C) Claim 7 recites "optionally followed by a concentration stage". Claim 18 recites "particularly by an ultrafiltration or affinity chromatography step". The phrases "optionally" and "particularly" render the claims indefinite because it is unclear whether the limitation(s) following the phrase are part of the claimed invention. See MPEP § 2173.05(d).

(D) Claims 23 and 24 provide for the use of anion exchange and affinity chromatography for the preparation or purification of membrane vesicles, but since neither claim has set forth any steps involved in the method/process, it is unclear what method/process applicant is intending to encompass. A claim is indefinite where it merely recites a use without any active, positive steps delimiting how this use is actually practiced.

Claim Rejections - 35 USC § 101

6. 35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

7. Claims 23 and 24 are rejected under 35 U.S.C. 101 because the claimed recitation of a use, without setting forth any steps involved in the process, results in an improper definition of a process, i.e., results in a claim which is not a proper process claim under 35 U.S.C. 101. See for example Ex parte Dunki, 153 USPQ 678 (Bd.App. 1967) and Clinical Products, Ltd. v. Brenner, 255 F. Supp. 131, 149 USPQ 475 (D.D.C. 1966).

Claim Rejections - 35 USC § 102

8. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless -
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

9. Claims 5 and 7 are rejected under 35 U.S.C. 102(b) as being anticipated by the abstract of Michaelson et al (Monographs in Neural Sciences, 1980, Vol. 7, pp. 19-29) as evidenced by Alberts et al (Molecular Biology of the Cell, 1989, pp. 1077-1079). Claim 5 is drawn to a process of preparing membrane vesicles from a biological sample, characterized in that it comprises at least treatment of the sample to prepare a sample enriched with membrane vesicles and a gel-permeation chromatography treatment of the sample. Claim 7 embodies the method of claim 5 wherein the enrichment step comprises a clarification stage optionally followed by a concentration stage.

The abstract of Michaelson discloses a method of purifying synaptic vesicles comprising differential and density gradient centrifugation and gel permeation (lines 5-6).

The instant specification states on page 1, lines 8-12, that particular membrane vesicles are more specifically obtained from intracellular compartments through fusion with the plasma membrane resulting in release in biological fluids. Alberts et al disclose that Ca+2 entry into the axon terminal of a neuromuscular junction results in the fusion of synaptic vesicles with the presynaptic membrane. Alberts et al discloses that these vesicles then release their contents into the synaptic cleft. Thus it appears that synaptic vesicles are consistent with membrane vesicles as set forth in the specification.

The specification (page 11, lines 12-14) states "the enrichment step comprises (i) the elimination of cells and/or cell debris (clarification)", therefore the differential centrifugation step disclosed by the abstract of Michaelson constitutes clarification, fulfilling the specific embodiment of claim 7.

10. Claims 5 and 7 are rejected under 35 U.S.C. 102(b) as being anticipated by Dubinsky et al (American Journal of Physiology, 1986, Vol. 251, pp. C713-C720) as evidenced by Langridge-Smith et al (Biochimica et Biophysica Acta, 1984, Vol. 777, pp. 84-92) and Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103)

The specific embodiments of the claims are set forth above.

Dubinsky et al disclose the preparation of membrane vesicles by a previously published procedure of Langridge-Smith et al (Biochimica et Biophysica Acta, 1984, Vol. 777, pp. 84-92) (page C714, first column, first sentence under the heading "Tracheal apical membrane preparation"). Langridge-Smith et al disclose a method for preparing a sample enriched with membrane vesicles comprising homogenization of tracheal epithelial scrapings, followed by Mg+2 precipitation and differential centrifugation (page 85, second column, under the heading

"Preparation of apical membrane vesicles"). Thus the Mg+2 precipitation step and the differential centrifugation meets the specific embodiment of claim 7 drawn to a "clarification stage". Dubinsky et al disclose a process of preparing membrane vesicles comprising passing said enriched vesicle preparation through a Sephadex G-50 column (abstract, lines 8-10 and page 85, second column, lines 12-18). Gordon (page 102, lines 5-7 and 13-18) discloses that the use of Sephadex to separate substrates based on size is described as "gel permeation" chromatography.

11. Claims 5 and 7 are rejected under 35 U.S.C. 102(b) as being anticipated Von der Decken (European Journal of Biochemistry, 1968, Vol. 4, pp. 87-94) as evidenced by Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and Smith et al (Oxford Dictinary of Biochemistry and Molecular Biology, 1997, page 419) and the abstract of Nishino et al (Archives of Biochemistry and Biophysics, 2000, Vol. 374, pp. 293-298) and the abstract of Tanaka et al (Journal of Biological Chemistry, 1987, Vol. 262, pp. 1374-1381) and the abstract of Seeger (Z. Krebsforsch, 1950, Vol. 57, pp. 113-120). The specific embodiments of claims 5 and 7 are set forth above.

Von der Decken discloses a process for preparing an enriched sample of microsomes comprising centrifugation of homogenized liver to obtain a supernatant, followed by layering of said supernatant onto a sucrose layer and further centrifugation to obtain a clear supernatant and a microsomal pellet (page 88, first column, first paragraph under the heading "Preparation of Microsomes, Ribosomes and Cell Sap"). Thus the centrifugation of the homogenized liver suspension fulfills the specific embodiment of claim 7 drawn to a clarification stage. Von der Decken discloses that the enriched sample of microsomes was further purified by passing through a column of Sephadex G-25 (abstract, lines 5-6 and page 88, under the heading "Molecular Sieving through Sephadex G-25").

The specification defines membrane vesicles as vesicles composed of a lipid bilayer containing a cytosolic fraction (page 1, lines 6-8).

Smith et al define a microsome as a vesicle formed from disrupted membranes of the endoplasmic reticulum and the plasma membrane. The abstract of Nishino et al states (last sentence) that the microsomal membrane is composed of a lipid bilayer. The abstract of Seeger defined cytoplasmic granules as comprising microsomes, and the abstract of Tanaka et al describes a cytoplasmic surface within a microsomal sphere. Thus, microsomes are vesicular structures which possess a lipid bilayer and contain a cytosolic fraction.

Gordon (page 102, lines 5-7 and 13-18) discloses that the use of Sephadex to separate substrates based on size is described as "gel permeation" chromatography.

Because microsomes fulfill the definition of membrane vesicles as set forth in the specification, and because the use of Sephadex columns are synonymous with gel permeation chromatography, it is concluded that Von der Decken discloses all of the specific embodiments of claims 5 and 7.

12. Claims 1, 2, 5, 7 and 23 are rejected under 35 U.S.C. 102(b) as being anticipated by Vaandrager et al (Biochimica et Biophysica Acta, 1988, Vol. 939, pp. 305-314) as evidenced by the Sigma Catalog, (1997, page 1801). Claim 1 is drawn to a process of preparing membrane vesicles from a biological sample, characterized in that it comprises at least one anion exchange chromatography treatment of the sample. Claim 2 embodies the method of claim 1 wherein it comprises at least one strong anion exchange chromatography step. Claim 5 is drawn to a process of preparing membrane vesicles from a biological sample characterized in that it comprises at least treatment of the sample to prepare a sample enriched with membrane vesicles, and anion-exchange chromatography treatment of the sample. Claim 7 embodies the method of claim 5 wherein the enrichment step comprises a clarification stage optionally followed by a

concentration stage. Claim 23 is drawn to the use of anion-exchange chromatography for the preparation of membrane vesicles.

Vaandrager et al disclose a process of preparing a sample enriched in membrane vesicles comprising differential Mg+2 precipitation (page 306, second column, lines 6-10 under the heading "Methods"). Thus the Mg+2 precipitation step, as it inherently comprises differential centrifugation, meets the specific embodiment of claim 7 drawn to a "clarification stage". Vaandrager et al disclose that the vesicle suspension was then passed through an anion-exchange column (abstract, lines 2-5 and page 306, second column, third paragraph). The anion exchange column used by Vaandrager et al is a strong anion exchanger as evidenced by page 1801 of the Sigma Catalog,(line 8 under "Dowex 1 Strongly Basic Anion Exchangers").

13. Claims 1, 5, 7 and 23 are rejected under 35 U.S.C. 102(b) as being anticipated by Denning et al (Journal of Protozoology, 1989, vol. 36, pp. 334-340). The embodiments of the claims are set forth in section 12, above.

Denning et al disclose a process for preparing clathrin-coated vesicles comprising centrifugation of a suspension of disrupted C reinhardtii cells to form a microsomal pellet (page 334, column 2, under the heading "Microsomal pellet preparation"), thus fulfilling the specific embodiment of claim 7 drawn to clarification. Denning et al disclose that the microsomes were further fractionated by sucrose gradient centrifugation and anion exchange chromatography on DEAE Biogel-A (page 334, column 2, under the headings, "Sucrose step gradients" and "DEAE-column chromatography" and abstract, line 2-4). Thus Denning et al discloses a process of preparing membrane vesicles comprising treating the sample to prepare enriched membrane vesicles by creating a suspension of disrupted cells and treatment of the sample by anion exchange chromatography.

14. Claims 1, 3, 5-7, 11, 12 and 23 are rejected under 35 U.S.C. 102(b) as being anticipated by Feldman et al (PNAS, 1987, vol. 84, pp. 6775-6779) as evidenced by the Sigma Catalog (1997, page 1815) and Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and the abstract of Reuveny et al (Developments in Biological Standardization, 1980, vol. 46, pp. 137-145) and Abbas et al (Cellular and Molecular Immunology, (text), 1991, page 17) and Raposo et al (Journal of Experimental Medicine, 1996, Vol. 183, pp. 1161-1172, reference AD of the I.D.S. submitted July 26, 2001). The embodiments of claims 1, 5, 7 and 23 are set forth above. Claim 3 is drawn to the method of claim 1 in that it comprises at least one anion exchange and gel permeation chromatography steps. Claim 6 is drawn to the process according to claim 5 characterized in that it comprises the culture of membrane vesicle (e.g. exosome) producing cells under conditions enabling release of the vesicles; a membrane vesicle enrichment step; and, an anion exchange and/or gel permeation chromatography treatment of the sample. Claim 7 is drawn to the process of claim 5 or 6 wherein the enrichment step comprises a clarification stage. Claim 11 is drawn to a process for preparing membrane vesicles characterized in that it comprises the culture of a population of membrane vesicle producing cells (e.g. exosomes) under conditions enabling the release of vesicles; the treatment of the culture supernatant with at least one ultrafiltration or affinity chromatography step to produce a sample enriched with membrane vesicles (e.g. with exosomes); and an anion exchange chromatography and/or gel permeation chromatography treatment step of the biological sample. Claim 12 embodies the process of claim 11 further comprising a filtration step of the treated preparation.

Feldman et al disclose a process of preparing membrane vesicles comprising the culturing of non-adherent lymphocytes (page 6779, second column lines 8-12). Feldman et al disclose that membrane derived vesicles are shed into the culture medium (page 6779, last paragraph, lines 7-10). Feldman et al disclose that lymphocyte culture medium was fractionated by centrifugation into membrane vesicle containing pellets (page 6775, second column, lines 22-24) thus fulfilling

the specific embodiment of claim 7 drawn to a clarification stage, as centrifugation of a sample results in clarification of said sample. Feldman et al disclose that vesicle fractions from the lymphocyte culture medium were applied to Sephacryl S-300 columns (page 6776, first column, under the heading "Chromatographic Fractionation of BPA", lines 5-8). The Sigma Catalog (page 1815) discloses that Sephacryl S-300 is a gel filtration medium. Gordon describes gel filtration to be equivalent to gel permeation (page 102, lines 15-18). Feldman et al disclose that fractions eluting from the Sephacryl S-300 which exhibited erythroid burst promoting activity in culture were pooled and concentrated by ultrafiltration (page 6776, first column, under the heading "Chromatographic Fractionation of BPA", lines 9-12). Thus, the disclosure of Feldman et al fulfills the specific embodiments of claims 5(b and c), 6(a, b and c), 11 (a and b), with regard to ultrafiltration and gel permeation chromatography treatment of the biological sample. Feldman et al further disclose the separation of the vesicle fraction by DE-52, DEAE-cellulose (page 6776, first column, under the heading "Chromatographic Fractionation of BPA" lines 5-15). The abstract of Reuveny et al defines DEAE-cellulose DE-52 as an anion exchanger. Thus the disclosure of Feldman et al fulfills the specific embodiments of claims 1, 3, 5(c), 6(c),11(c) and 23, as drawn to anion exchange chromatography. Feldman et al further disclose that the eluent of the DE-52 column was applied to a hydroxyapatite column (page 6776, first column, under the heading "Chromatographic Fractionation of BPA" lines 22-26) fulfilling the specific embodiment of claim 12 comprising an additional filtration step after the anion exchange chromatography treatment step.

Claims 6 and 11 recite "(...exosomes)". For the reasons stated in the rejection under 112, second paragraph above, it is not clear if this is a specific embodiment of the claim. However, in the event that the claims are amended to be clearly drawn to "exosomes", it appears that the membrane vesicles shed into the culture medium by non-adherent lymphocytes are exosomes. Raposo et al disclose that B lymphocytes secrete vesicles termed exosomes into the cell culture medium (page 1162, first column, lines 18-26 and under the heading "Isolation and Purification of

Exosomes", lines 1-8). Feldman et al teach that non-adherent lymphocytes obtained from plateletpheresis residues were incubated in culture to produce conditioned medium that containing membrane vesicles (page 6775, second column, under the heading "Preparation of Lymphocyte Plasma Membranes and Conditioned Medium"). Abbas et al disclose that 10-15% of lymphocytes found in blood are B lymphocytes (Table 2-2). It is reasonable to conclude that the membrane vesicles isolated by Feldman et al included exosomes, as the non-adherent lymphocytes cultured by Feldman et al would have contained B lymphocytes.

Claim Rejections - 35 USC § 103

15. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

16. Claims 1-3, 5-7, 11, 12, 17-19 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Feldman et al (PNAS, 1987, vol. 84, pp. 6775-6779) as evidenced by the Sigma Catalog (1997, page 1815) and Gordon (Electrophoresis and Chromatography, In: The

Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and the abstract of Reuveny et al (Developments in Biological Standardization, 1980, vol. 46, pp. 137-145) and Abbas et al (Cellular and Molecular Immunology, (text), 1991, page 17) and Raposo et al (Journal of Experimental Medicine, 1996, Vol. 183, pp. 1161-1172, reference AD of the I.D.S. submitted July 26, 2001), in view of Zitvogel et al (WO 99/03499, reference AC of the I.D.S. filed July 26, 2001) and Thiery et al (Journal of Cell Biology, 1999, vol. 147, pp. 599-610) and Chen et al (Journal of Chromatography, 1995, vol. 666, pp. 178-182) and Pharmacia Biotechnology (Ion Exchange Chromatography, Principles and Methods, 1991, 3rd edition, page 34).

Feldman et al as evidenced by the Sigma Catalog and Gordon and the abstract of Reuveny et al and Abbas et al and Raposo et al anticipate claims 1, 3, 5-7, 11, 12 and 23 for the reasons set forth in section 14, above.

Claim 2 is drawn to the process according to claim 1 characterized in that it comprises at least one strong anion exchange chromatography step. Claim 17 is drawn to a process of preparing membrane vesicles comprising (a) obtaining a population of dendritic cells, (b) culturing the dendritic cells under conditions enabling the production of membrane vesicles, and (c) purifying the membrane vesicles using at least one anion exchange chromatography treatment. Claim 18 is drawn to a process of preparing membrane vesicles comprising (a) obtaining a population of dendritic cells, (b) culturing the dendritic cells under conditions enabling the production of membrane vesicles, (c) treating the culture supernatant to produce a biological sample enriched with membrane vesicles by an ultrafiltration step and (d) purifying the membrane vesicles using a process comprising at least one anion exchange chromatography step. Claim 19 is drawn to a process according to claims 17 or 18 wherein the dendritic cells are obtained from bone marrow or peripheral blood.

Feldman et al teach the use of DEAE-cellulose (DE-52) for the preparation and purification of membrane vesicles shed from cultured lymphocytes. DEAE-cellulose is a tertiary

amine and according to page 6, lines 27-30 of the instant specification, a weak anion-exchanger; therefore, Feldman et al do not teach the specific embodiment of claim 2 drawn to a strong anion exchanger, or the specific embodiments of claims 17 and 18 drawn to dendritic cells.

Zitvogel et al (1999) teach dexosomes as membrane vesicles derived from dendritic cells (abstract and title). Zitvogel et al suggest ion exchange chromatography, gel permeation chromatography and ultrafiltration (nanofiltration) for the purification of dexosomes (page 27, lines 15-20). Zitvogel et al teach that due to the nature of the dexosome lipid membrane, ion exchange chromatography is especially of interest.

Zitvogel et al do not specifically teach anion exchange as the specific type of ion exchange chromatography.

Thiery et al teach conditions for producing dexosomes from dendritic cells (page 603, under the heading "Exosomes production during DC maturation"). Thiery et al teach dendritic cells obtained from bone marrow (page 600, first column, last two lines) and human monocyte-derived dendritic cells (page 603, second column, last sentence), thus fulfilling the specific embodiments of claims 11 (a), 17 (a and b) and 18 (a and b)drawn to culturing a population of exosome producing cells and culturing a population of dendritic cells under conditions enabling the production of membrane vesicles as well as the specific embodiments of claim 19 drawn to dendritic cells obtained from bone marrow or peripheral blood, as monocytes having the potential to give rise to dendritic cells would be in peripheral blood. Thiery et al teach a clarification step using protein G-Sepharose (page 601, under the heading "Metabolic Labeling of Cells and Exosomes, lines 13-17), thus fulfilling the specific embodiment of claim 7, drawn to a clarification step.

Thiery et al do not teach anion exchange chromatography, gel permeation or ultrafiltration to isolate or purify the dendritic cell exosomes. However, Thiery et al teach that exosomal membranes have exposed phosphatidylserine residues (page 607, last paragraph, lines 7-9).

Chen et al teach that polyunsaturated acid-containing molecular species of phosphatidylserine can be isolated from bovine brain extract by means of anion exchange chromatography without loss of the polyunsaturated moiety (abstract). Chen et al teach that Q-Sepharose was the anion exchange resin (page 179, first column under the heading "Chemicals"). Pharmacia Biotechnology (page 34, second paragraph) identifies Q Sepharose as a strong anion exchanger.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute dendritic cell derived exosomes for the vesicles shed by B-lymphocytes in the methods of preparation and purification as taught by Feldman et al. Further, it would be obvious to purify the dendritic cell exosomes by a strong anion exchanger such as Q-Sepharose as taught by Chen. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the suggestion of Zitvogel et al that dendritic cell derived exosomes be purified by gel permeation chromatography, ultrafiltration and in particular by ion-exchange chromatography due to the nature of the dendritic cell exosomal membrane; the teaching of Thiery et al on the presence of exposed phosphatidylserine in the membrane of exosomes; and the teachings of Chen et al on a process for purifying molecular species comprising phosphatidylserine by the use of anion exchange chromatography on Q Sepharose, a strong anion exchanger.

17. Claims 1-3, 5-7, 11, 12, 17-19, 23 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Feldman et al and the Sigma Catalog (1997, page 1815) and Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and the abstract of Reuveny et al (Developments in Biological Standardization, 1980, vol. 46, pp. 137-145) and Abbas et al (Cellular and Molecular Immunology, (text), 1991, page 17) and Raposo et al (Journal of Experimental Medicine, 1996, Vol. 183, pp. 1161-1172, reference AD of the I.D.S. submitted

July 26, 2001) and Zitvogel et al (WO 99/03499, reference AC of the I.D.S. filed July 26, 2001) and Thiery et al (Journal of Cell Biology, 1999, vol. 147, pp. 599-610) and Chen et al (Journal of Chromatography, 1995, vol. 666, pp. 178-182) and Pharmacia Biotechnology (Ion Exchange Chromatography, Principles and Methods, 1991, 3rd edition, page 34), as applied to claims 1-3, 5-7, 11, 12, 17-19 and 23 above, and further in view of Zitvogel et al (Nature Medicine, 1998, Vol. 4, pp. 594-600) and Amigorena (Hematology and Cell Therapy, 1997, Vol. 39, pp. 87-89) and Ogle et al (U.S. 6,165,785). The combination of Feldman et al and the Sigma Catalog and Gordon and the abstract of Reuveny et al and Abbas et al and Raposo et al and Zitvogel et al (1999) and Thiery et al and Chen et al and Pharmacia Biotechnology renders obvious claims 1-3, 5-7, 11, 12, 17-19 and 23 for the reasons set forth in section 16, above. Claim 24 is drawn to the use of affinity chromatography for the preparation or purification of membrane vesicles. Additionally, claim 11, part b, and claim 18, part (c) are also drawn to affinity chromatography.

Thiery et al teach that exosomes produced by dendritic cells exposed to tumor-derived antigenic peptides induce potent immune responses, such as cytotoxic T-lymphocytes, leading to the regression of established tumors in mice (page 600, first column, second full paragraph). Thiery et al teach that dendritic cell-derived exosomes have both MHC class II and MHC class I molecules. Thiery et al teach that batches of exosomes derived from culture of murine dendritic cells were contaminated by bovine exosomes present in the fetal calf serum used for the culture medium (page 600, second column, second paragraph under the heading "Exosome Purification"). Thiery et al teach that the actual presence of murine derived dendritic cell exosomes was demonstrated by immunoprecipitation. The legend for figure 7 states that exosomes were immunoprecipitated with antibodies specific to MHC II.

Zitvogel et al (1998) teach that administration of dendritic cell derived exosomes loaded with tumor peptides results in tumor growth delay or tumor eradication when administered to mice bearing tumors. Zitvogel et al teach that these exosomes contained syngeneic MHC molecules (page 595, second column, last line to page 596, first column line 1). Zitvogel et al

teach that the administration of exosomes bearing allogenic MHC molecules did not induce significant anti-tumor responses (page 597, first column, lines 13-16).

Amigorena teaches that dendritic cell exosomes bear CD63 and CD82 markers absent from the dendritic cell surface (page 87, second colum, lines 1-3).

Olge et al teach that enriched populations can be obtained from mixed cell suspensions by positive selection (column 21, lines 16-17) and that the technology for capturing specific cells on affinity materials is well known in the art. Ogle et al further teach that procedures for separation of cells may include affinity chromatography.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to purify exosomes or dexosomes by affinity chromatography using an antibody directed toward the CD63 or CD82 molecule of the syngenic dendritic exosome. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Thiery et al on the contamination of dendritic cell exosomes with bovine exosomes, the teaching of Zitvogel et al on the usefulness of syngenic, versus allogenic, dendritic exosomes in the treatment of tumors, the teachings of Amigorena on the presence of CD63 and CD82 exclusively on the surface of dendritic cell exosomes, the teachings of Olge et al on the general utility of separations using affinity materials. One of skill in the art would be motivated to use affinity chromatography in order to remove contaminating bovine exosomes because the contaminated preparations would evoke an anti-bovine response when administered in vivo and because the contaminating bovine exosomes would not present tumor-derived peptides in the context of syngeneic MHC molecules which are taught by Zitvogel et al to be necessary to evoke an anti-tumor response.

18. Claims 1, 3, 5-7, 11, 12, 23 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Feldman et al (PNAS, 1987, vol. 84, pp. 6775-6779) and the Sigma Catalog (1997, page 1815) and Gordon (Electrophoresis and Chromatography, In: The Origins of Modern

Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and the abstract of Reuveny et al (Developments in Biological Standardization, 1980, vol. 46, pp. 137-145) and Abbas et al (Cellular and Molecular Immunology, (text), 1991, page 17) and Raposo et al (Journal of Experimental Medicine, 1996, Vol. 183, pp. 1161-1172, reference AD of the I.D.S. filed July 26, 2001) in view of Olge et al (U.S. 6,165,785). Feldman et al as evidenced by the Sigma Catalog and Gordon and the abstract of Reuveny et al and Abbas et al and Raposo et al teach the specific embodiments of claims 1, 3, 5-7, 11, 12 and 23 for the reasons stated above.

Claim 24 is drawn to the use of affinity chromatography for the preparation or purification of membrane vesicles. Additionally, claim 11, part b is also drawn to affinity chromatography.

Feldman et al further teach that an anti-membrane IgG bound to plasma membranes fractionated over from Sephacryl 300, hydroxyapatite and DE-25 (page 6778, first column, lines 20-27 and the Legend for figure 4). Feldman et al do not teach the use of the anti-membrane IgG conjugated to a solid support for an affinity chromatography step.

Olge et al teach that enriched populations can be obtained from mixed cell suspensions by positive selection (column 21, lines 16-17) and that the technology for capturing specific cells on affinity materials is well known in the art. Ogle et al further teach that procedures for separation of cells may include affinity chromatography.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to add a separation stage based on affinity chromatography to the process of preparing membrane vesicles comprising anion exchange chromatography, gel permeation chromatography and ultrafiltration as taught by Feldman et al. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Ogle et al on the separation of cells based on affinity chromatography and the teachings of Feldman et al on the binding of an anti-membrane antibody to the plasma membranes isolated by anion exchange and gel permeation chromatography.

19. Applicant cannot rely upon the foreign priority papers to overcome the rejections of sections 16 and 17 because a translation of said papers has not been made of record in accordance with 37 CFR 1.55. See MPEP § 201.15.

20. All claims are rejected.

Conclusion

21. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Canella whose telephone number is (703) 308-8362. The examiner can normally be reached on Monday through Friday from 8:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached on (703) 308-3995. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

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